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SHORT COMMUNICATION

Distribution of gymnemic acid in various organs of *Gymnema sylvestre*

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Abstract: The gymnemic acid contents in various organs of *Gymnema sylvestre* were investigated by High Performance Liquid Chromatography (HPLC) method. The results shows that the content of gymnemic acid in various organs, obviously different, was 54.29, 31.66, 28.82, 27.67, 25.39, 20.56 and 1.31 mg·g^{-1} DW in shoot tips, flowers, nodes, leaves, internodes, roots and seeds, respectively. The highest gymnemic acid content (54.29 mg·g^{-1} DW) was found in shoot tip, 1.96 fold higher than that in leaves (27.67 mg·g^{-1} DW). Maximum quantity of gymnemic acid (35.39 mg·g^{-1} DW) was observed in the young leaves, which was 1.52 times higher than that in old leaves (23.07 mg·g^{-1} DW). The content of gymnemic acid in young, middle and old internodes was 26.47, 25.77 and 23.94 mg·g^{-1} DW, respectively, all lower than that in leaves (27.67 mg·g^{-1} DW), whereas the content of gymnemic acid in young, middle and old nodes was 27.96, 28.81 and 29.66 mg·g^{-1} DW, respectively, all higher than that in leaves. The study provides the scientific evidences for the rational development and utilization of *Gymnema sylvestre* resources, since over exploitation of natural stands has caused depletion of these plants in nature.

Keywords: gymnemic acid; *Gymnema sylvestre*; high performance liquid chromatography

Introduction

Higher plants accumulate various secondary metabolites to avoid plant and environment interaction. These secondary metabolites are extremely useful to human beings as industrial and biomedical products. Accumulation of secondary metabolite is not uniform though out the plant body and it is dependent on accessibility of the sites of precursor molecules. Exposed external tissues are usually better defended and accumulate higher amounts of secondary metabolites than internal tissues in roots, stems, leaves, seeds, bulbs and tubers and in fruits secondary metabolites are neutralized during maturation so the fruits will become attractive to dispersers. In order to utilize the plants or plant parts for medicinal purposes, it is essential to know the distribution secondary metabolites (Zhao et al. 2007), which enables to choose the right organs and to obtain good resources for extraction.

Gymnema sylvestre belongs to the Asclepiadaceae family and the plant is considered to be a good source of a large number of bioactive substances. It is a vulnerable medicinal species, being a slow growing, perennial, woody climber found in India and the southwestern region of China. It has a reputation as a traditional remedy to control diabetes mellitus (Shimizu et al. 1996; Shimizu et al.1997). In addition, the leaves of this plant, popularly known as Madhunashni or Gur-mar in India, are used for inhibiting the taste of sweetness (Liu et al. 1992). A number of saponins such as gymnemic acid, deacyl gymnemic acid, gymnemagnenin (Subbarao et al. 1971; Gooper 1887), 23-hydroxylnogispinogenin, and gymnestrogenin have been purified (Sahu et al. 1996; Yoshikawa et al. 1992; Yoshikawa et al. 1997) from *Gymnema sylvestre*. The leaf extract from this plant is used as stomachic, stimulant, laxative, diuretic, anti-sweetner, antibacterial (Yoshikawa et al. 1992), antiviral and anti-inflammatory (Satdive et al. 2003) activities. HPLC methods have already been reported for the estimation of gymnemic acid in *Gymnema sylvestre* (Diwan et al. 1995; Yokota et al. 1994). The quantity of gymnemic acid, the active principle in *Gymnema* leaves is, however, variable among accessions from different eco-climatic regions (Yokota et al. 1994). Considerable variations also exist among the morphological traits of *Gymnema* accessions from Tamil Nadu and Kerala (Thamburaj et al. 1996). In this study, a detail investigation of the distribution of gymnemic acid content in various organs of *Gymnema sylvestre* was carried out. In particular, the contents of gymnemic acid in various organs like leaves, shoot tips, internodes, nodes, roots, flowers and seeds of *Gymnema sylvestre* were determined and compared.

Materials and methods

Plant material

Gymnema sylvestre samples (shoot tips, leaves, nodes, internodes, flowers, roots and seeds) were collected from the Botanical Garden, Karnatak University, Dharwad, India for the determination of gymnemic acid contents. The second young leaf - 18 mm × 7

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mm, petiole- 3 mm, fifth middle leaf- 32 mm ×22 mm, petiole- 4 mm) and ninth old leaf- 49 mm ×28 mm, petiole- 6 mm were collected. Each of these leaves was cut into four parts (Leaf apex, leaf middle, leaf base and petiole) and separated. Young internodes (between the first and second node), middle internodes (between the fourth and fifth node) and old internodes (between the eight and ninth node) were excised as shown in Fig.1. The second, fifth and ninth node were separated. The shoot tip, mature flowers, roots and seeds were also collected. The above samples were shadily dried, ground to a fine powder, sieved through a 20-μm stainless sieve (Sigma, USA) and used for extraction and estimation of gymnemic acid.

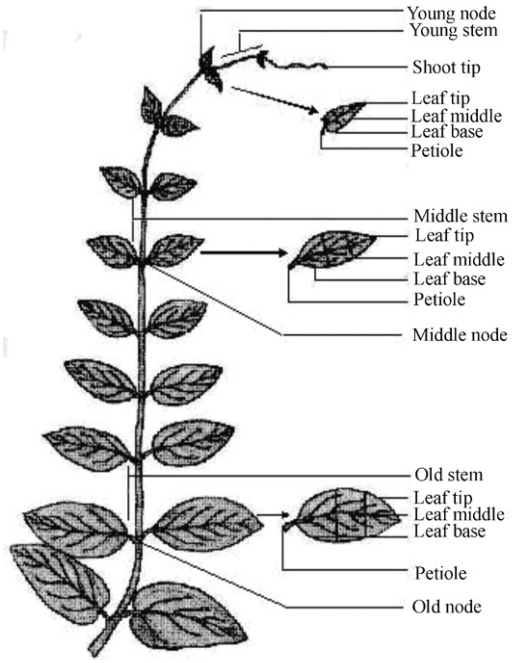

Fig. 1 Schematic picture of *Gymnema sylvestre* organs used for the analysis of gymnemic acid contents

Extraction of gymnemic acid

We put 500 mg of sample into a 500-mL round bottom flask and added 50 mL of extraction solvent (volume ratio of methanol to water is 1:1) and 10 mL of 11% potassium hydroxide solution. The mixture was refluxed for an hour. The concentrated HCl of 9 mL was added and refluxed again for one hour. The mixture was cooled to room temperature. The extract was filtered through 0.45-μm nylon filter (Millipore), the volume was made up to 100 mL with extraction solvent, and the clean supernatant was used for HPLC analysis.

Determination of gymnemic acid II by HPLC

The analytical HPLC experiments were performed with a Shimadzu high performance liquid chromatography equipped with variable wave length detector operating at 210 nm (SPD-10AVP, LC-10ATVP). Separations were carried out with Luna C-18 (150 mm × 3 mm, 5 μm) column with a column temperature of 26ºC. The mobile phase was acetonitrile (A) and water (B) (80A:20B) with elution rate of 1 mL/min. Gymnemagenin standard was obtained from ChromaDex (Laguna Hills, CA, USA). Validation of quantitative method was performed with samples consisting of five replicates of 20 μL each. The chromatogram of gymnemic acid in *Gymnema sylvestre* shoot tips was shown in Fig. 2 with a retention time of 1.83 min. The conversion of gymnemagenin to gymnemic acid was done using the equation as follows:

$$C = X\,(809.0/\,506.7) \quad (1)$$

where, C is the content of gymnemic acid in the sample; X is the content of gymnemagenin present in the sample, 506.7 is the molecular weight of gymnemagenin, and 809.0 is the molecular weight of gymnemic acid.

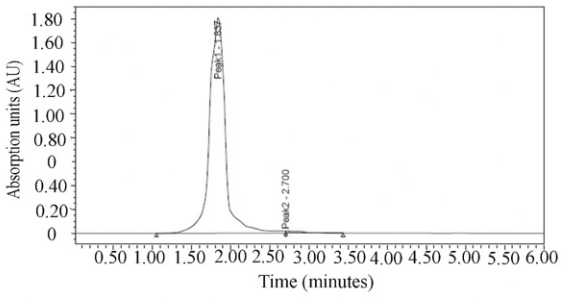

Fig. 2 HPLC chromatogram of gymnemic acid contents in *Gymnema sylvestre* shoot tip; x-coordinate is Time (minutes) and y-coordinate is absorption (absorption units)

Results and discussion

The contents of gymnemic acid II were significantly different from the different organs of *Gymnema sylvestre* (Fig. 3). The maximum content of gymnemic acid was obtained in shoot tips (54.29 mg·g^{-1} DW), followed by the leaves, flowers, nodes, internodes and roots (27.67, 31.66, 28.81, 25.39 and 20.56 mg·g^{-1} DW, respectively). Whereas seeds, accumulated lower concentration of gymnemic acid (1.31 mg·g^{-1} DW), the content of gymnemic acid was 1.71, 1.88, 1.96, 2.13, 2.64 and 41.39 times higher in shoot tips than that in flowers, nodes, leaves, internodes, roots and seeds, respectively. Many literatures reported that the leaves of *Gymnema sylvestre* were the major resource for the production gymnemic acid (Gooper 1887; Yokota et al. 1994).

The above results show that gymnemic acid was not only present in the leaves but also in shoot tips, internodes, nodes, flowers, roots, and seeds. The contents of gymnemic acid in leaves were significantly different and the young leaves had higher content (35.39 mg·g^{-1} DW) than middle (24.55 mg·g^{-1} DW) and old leaves (23.07 mg·g^{-1} DW), thus the young leaves contained 1.52 fold higher gymnemic acid content than that of old leaves.

The content of gymnemic acid in various parts of middle leaves also varied considerably and leaf apex had higher content (26.84 mg·g^{-1} DW) than leaf middle (25.26 mg·g^{-1} DW), leaf base (23.7 mg·g^{-1} DW) and petiole (22.39 mg·g^{-1} DW). These values proved that the higher concentration of gymnemic acid was accumulated in tip of the leaves, compared to other parts of the leaves. The content of gymnemic acid in young, middle and

old internodes was 26.47, 25.77 and 23.94 mg·g^{-1} DW, respectively, which were lower than that in leaves (27.67 mg·g^{-1} DW). The content of gymnemic acid in young, middle and old node was 27.96, 28.81 and 29.66 mg·g^{-1} DW, respectively, which were higher than that in leaves (27.67 mg·g^{-1} DW) (Table 1).

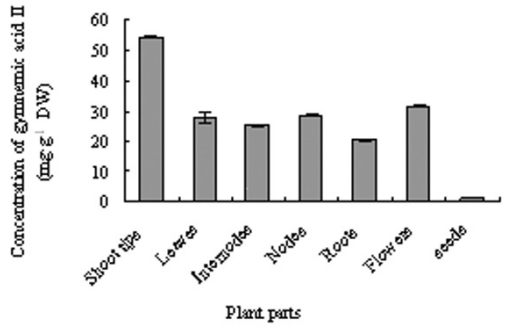

Fig. 3 Distribution of gymnemic acid in *Gymnema sylvestre* (Values are mean ± SE of 5 replicates; data are pooled results of different aged organs)

Table 1. Contents of gymnemic acid in various organs and tissues of *Gymnema sylvestre*

Samples		Gymnemic acid content (mg·g^{-1} DW ± SE)
(a) Shoot tip		54.29 ± 0.39
(b) Leaves		
Young leaf	Whole leaf(18 mm×7 mm)	35.39 ± 0.31
	Leaf tip	38.25 ± 0.42
	Leaf middle	36.96 ± 0.18
	Leaf base	35.46 ± 0.33
	Leaf petiole (3mm)	30.94 ± 0.32
Middle leaf	Whole leaf (32 mm×22 mm)	24.55 ± 0.27
	Leaf tip	26.84 ± 0.13
	Leaf middle	25.26 ± 0.36
	Leaf base	23.70 ± 0.24
	Leaf petiole (4mm)	22.39 ± 0.35
Old lief tip	Whole leaf (49 mm×28 mm)	23.07 ± 0.40
	Leaf tip	24.60 ± 0.20
	Leaf middle	23.50 ± 0.32
	Leaf base	22.74 ± 0.67
	Leaf petiole (6 mm)	21.45 ± 0.41
(c) Node		
Young node		27.96 ± 0.22
Middle node		28.81 ± 0.40
Old node		29.66 ± 0.66
(d) Internode		
Young internode		26.47 ± 0.31
Middle internode		25.77 ± 0.23
Old internode		23.94 ± 0.52
(e) Flower		31.66 ± 0.21
(f) Root		20.56 ± 0.16
(g) Seeds		1.31 ± 0.09

Notes: (a) Shoot tip; (b) young leaf - second leaf; middle leaf - fifth leaf; old Leaf - ninth leaf; (c) young node – second node, middle – fifth node, old node – ninth node; (d) young internodes - between first and second node; middle internodes - between fourth and fifth node, old internodes – between eighth and ninth node. SE- standard error (Values are mean ± SE of 5 replicates).

In the present study, it was observed that the maximum contents of gymnemic acid were accumulated in the shoot tips and fresh leaves, which are renewable source. The probable reasons for the enrichment of these metabolites in these parts may be attributed to environmental factors, genotypes, morphotypes or cultivation practices.

In conclusions, the content of gymnemic acid in shoot tip was higher than that in any other organs. In previous study, gymnemic acid was mainly produced from the leaves of *Gymnema sylvestre* (Gooper 1887; Yokota et al. 1994) and the rest of the plant was wasted. The present study proved that the internodes, roots and flowers are also a good resources for production of gymnemic acid. Thereby, the stalks, especially the top stalks are worthy to be the new resource for production of gymnemic acid. The leaves and stalks of *Gymnema sylvestre* will play an important role in the increase of the availability of raw material for pharmaceutical purpose.

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